

Oxidative stress and lipid per-oxidation with repeatedly heated mix vegetable oils in different doses in comparison with single time heated vegetable oils

Afshan Siddiq^{1*}, Gul Ambreen^{1,2}, Kashif Hussain², Sadia Ghousia Baig¹, Saira Saeed Khan¹, Muhammad Osama¹ and Rahila Ikram¹

¹Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

²Aga Khan University Hospitals, Karachi, Pakistan

Abstract: Cardiovascular diseases and cancer are the leading cause of death worldwide, changed lifestyle and eating habits are the major contributing factors. Daily consumption cooking oils is one of the nutritional sources in today's life. Oils are available in market in the blend of two or more oils to get the maximum health benefits. There are number of factors which decide the pathogenic or protective effects of these oils, like fatty acids (FAs) composition, duration and extent of thermal exposure, daily intake and consumption duration. While processing the food cooking oils are thermally oxidized, that exert deleterious health effects, when taken for long time. The present study designed to evaluate the lipid peroxidation and level of oxidative stress in rabbits treated with repeatedly heated mix vegetable oils, in low (L-RHMVO) and high doses (H-RHMVO) in comparison with single time heated olive (STH-OO), canola (STH-CO), sunflower (STH-SO) oils individually and in mixture (STH-MVO) collected from Karachi (Pakistan). Six groups of animals treated with all these processed oils for 16 weeks along with normal diet. Control group was kept on normal rabbit diet. Animal body and organ weight was recorded. Blood samples were collected to measure plasma Malondialdehyde (MDA), Homocysteine (H-Cys), Creatine phosphokinase (CPK), Lactate dehydrogenase (LDH), C-reactive protein (CRP) and lipid profile (TGs, Total-cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol). Statistically highly significant ($p < 0.005$) increased body and organ weight along with Total-cholesterol, TGs, LDL-cholesterol, VLDL-cholesterol, H-Cys, MDA, CPK, LDH & CRP and decreased HDL-cholesterol was found in H-RHMVO and L-RHMVO groups in dose dependent manner compared to control and single time heated oils groups. Among the single time heated oils STH-SO fed animals had significant ($p < 0.05$) increase in lipid peroxidation and oxidative stress parameters. STH-OO showed least variation from control with significant increase in HDL-cholesterol level. The finding of this study not only confirms health deleterious effect of vegetable oils when used in thermally oxidized condition but also suggests induced-metabolic changes with oxidative stress. So more advance studies simulating real-life exposure to multiple hazardous substances is required.

Keyword: Lipid per-oxidation, oxidative stress, repeatedly heated mix vegetable oils, Malondialdehyde

INTRODUCTION

About 31% of all global deaths were due to cardiovascular diseases (CVDs) in 2015, 6.7 million due to stroke and 7.4 million with coronary heart disease. In addition, about three quarters of all deaths due to CVDs were only reported from countries with low to middle income (<http://www.wcrf.org>). The South Asians population has the highest known rate of coronary artery disease (Nishtar, 2002). The ongoing cancer transition includes increases in cancers linked to a change of lifestyle (Bray *et al.*, 2012). Worldwide approximately 14.1 million cases of different malignancies were reported in 2012. The predicted addition is up to about twenty four million till 2035. Liver, colorectal & stomach cancers are among the top five frequently detected reasons of deaths due to cancer (<http://www.wcrf.org>). Sanitary lifestyle, overweight, use of alcohol, chain smoking, over intake of fried and meat products with reduced vegetable and fruits consumption are the attributing risk factors (Obloh *et al.*,

2014).

Vegetable Cooking oils are an essential portion of human diet being the major lipid source, which serve as energy source and integral of bio-membrane (Vaskova & Buckova, 2015) and hormonal building block. Cooking oils mostly consumed in cooking process to enhance palatability of the food (Warner, 2004), while cooking food products and oil get exposed to high heat, leading to oil oxidation (Obloh *et al.*, 2014). This is routine practice at homes in many countries (Falade & Obloh, 2015). At commercial level to minimize the cost and get maximum profit reuse of oil and addition of more oil of any type to get the sufficient quantity for frying without taking the used oil out of pan, is common practice in south Asian countries like Pakistan. Several studies have discovered and reported the harmful effect of thermal oxidation on cooking oils and processed food (Obloh *et al.*, 2014), but regardless of all that practice seems to continue.

*Corresponding author: e-mail: afshan@uok.edu.pk, gul.ambreen@aku.edu

Physicochemical characteristics of the cooking oils changes when heated beyond a certain limit (Obboh *et al.*, 2014; Falade & Obboh, 2015) as chain of several chemical reactions take place in moisture and air presence, oils degenerates and produce volatile substances, unwanted monomers, polymers and isomers and the free radical formation (Andrikopoulos *et al.*, 2002). Other influencing factors on the deterioration of cooking oils and processed food (Ganesan, 2017), are heating duration & temperature, FAs composition, ratio of different oils in the mixture, existence of antioxidants. In Pakistan most of the commercially available oils are the blend of two or more edible oils, appropriate ratio of different FAs is important to achieve maximum benefit from vegetable oils. High polyunsaturated FAs(PUFA) containing oils are easily oxidized (Vaskova & Buckova, 2015).

Several animal studies demonstrate that consumption of repeatedly heated vegetable oils (RHVOs) increased risk of CVDs like hypertension with reduced vasorelaxation responses (Owu *et al.*, 1997), endothelial malfunction (Lopez-Garcia *et al.*, 2005), lipid peroxidation (Adam *et al.*, 2008b), atherosclerosis (Adam *et al.*, 2008a), oxidative stress (Leong *et al.*, 2018), genotoxicity (Dung *et al.*, 2006) and carcinogenicity (Sinha *et al.*, 1999), deranged liver function (Owu *et al.*, 1998) and lower glucose absorption (Obembe *et al.*, 2010). On the other hand some evidences are there with non-significant deleterious impact of RHMVO in animals (Ribeiro *et al.*, 2010). But to our information no such effort has been reported till day to explore the injurious consequences of long term use of RHMVO available for common use in Karachi Pakistan, in particular.

The present study designed to evaluate the effect of RHMVO on rabbit's lipid peroxidation and oxidative stress in high and low doses in comparison with individual and mixture of single time heated vegetable oils.

MATERIAL AND METHOD

Oil samples preparation

Standard food grade Canola, sunflower and olive oils (7 Liter of each oil) were purchased from Karachi local market. Sufficient quantity of oil (4L from each oil) was separated and thermally treated above 300°C (beyond its smoke point) for 45 minutes with potatoes frying and then cooled to room temperature, labeled as single time heated Canola oil (STH-CO), single time heated Sunflower oil (STH-SO) and single time heated Olive oil (STH-OO). Then one liter from single time heated each oil was taken and mixed together and labeled as Single time Heated Mix Vegetable Oils (STH-MVO). Then remaining oils (3 Liter of each oil) were mixed together. For coming 9 days potatoes were fried at same temperature for 45 minutes and then cooled to room

temperature, without addition of fresh oil. At the end of 10th day (approximately 8.2L) viscous dark brown oil was obtained, to prevent photo degradation it was stored in amber color bottles and labeled as Repeatedly Heated Mix Vegetable Oils (RHMVO).

Experimental animals

Healthy male rabbits weighing between 1400-1500g were purchased from the department of pharmacology, university of Karachi, Pakistan. Rabbits were kept individually in wire topped steel cages with wooden bottoms, provided control conditions, temperature 23 ± 2 °C with 50-60% relative humidity for a period of 16 weeks with an estimated 12/12 hours light/dark photocycle. Animals were acclimatized for the period of seven days before starting experiment. Animal were handled throughout according to the Institutional animal ethical committee guidelines. All the groups' animals were fed as per the study design mentioned in study procedure. Oils daily doses were calculated on the basis of animal doses used in previous studies (Venkata and Subramanyam, 2016) and higher doses to mimic the high intake with fried food.

Experimental Procedure

The study was performed by randomly allocating rabbits in following seven treatment categories (n = 12; in each treated group).

- Unexposed control Rabbits (fresh hay and water only) = Normal diet
- Rabbits treated with single time heated Canola oil : fed on 1.5ml STH-CO + Normal diet
- Rabbits treated with single time heated Sunflower oil : fed on 1.5ml STH-SO + Normal diet
- Rabbits treated with single time heated Olive oil : fed on 1.5ml STH-OO + Normal diet
- Rabbits treated with single time heated mix vegetable oil: fed on 1.5ml STH-MVO + Normal diet
- Rabbits treated with Low dose Repeatedly Heated Mix Vegetable Oils (L-RHMVO): fed on 1.5ml RHMVO + Normal diet
- Rabbits treated with High dose Repeatedly Heated Mix Vegetable Oils (H-RHMVO): Fed on 3ml RHMVO + Normal diet

Oils were administered orally for 16 weeks. Morbidity and mortality was monitored. Animal's body weight was recorded at base line followed by weekly monitoring for 4 weeks then on week 8, 12 and 16. At the end of 16 weeks animals were sacrificed to get stomach, kidney, heart and liver for organ weight evaluation.

Blood sample collection for lipid profile

Samples of blood were collected from ear vein of animals in fasting state at 16th week completion. For hematological parameters study, blood volume of 2ml

was taken in EDTA.K3 tubes, 5 ml volume in gel tube to perform the biochemical assays for lipid profile. Promptly carried out the centrifugation of collected blood samples (at 3000 rpm; 15min duration) to isolate the serum. With the use of clean dry disposable plastic syringes the supernatant was parted and stored (at temperature of -18°C) and then used to analyze the plasma Lipid profile (TG, Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol), using analytical kits of RANDOX Laboratories Ltd. following the manufacturer's instructions.

Oxidative Stress and tissue damage parameters

Lipid peroxidation also measured by determining plasma MDA, following method by Ledwozyw *et al* (1986). The plasma CRP was measured by using ELISA kit (Abnova, Taipei, Taiwan), as per the manufacturers instruction for use. Commercially available kit was used to analyses LDH (BioVision, USA). The samples as well as standards were prepared as per manufacturer's method, following Hamsi MA *et al* (2015). To measures the Fasting plasma homocysteine concentrations we adopted the method described by Sheu WH *et al* (2000). CPK was measured by a commercially available kit from Beckman Coulter, Brea, USA, followed by manufacturer instructions.

STATISTICAL ANALYSIS

In our study the final data was accessible in the form of mean $\pm$ standard deviation (SD). Applied analysis of variance (ANOVA) followed by post hoc Tukey's Honest Significant Difference (HSD) test to find out statistical significance. The value $p < 0.05$ was taken as significant and $p < 0.005$ as highly significant. The data analysis of this study was performed using SPSS (Statistical Product and Service Solutions) software.

RESULTS

Gross observation of animals

No death and unexpected signs and symptoms noticed in experimental animals throughout 16 weeks study period with oral intake of any processed oil. Highly significant ($p < 0.005$) variation in body (fig. 1) and organ weight (liver, stomach, kidney and heart) (fig. 2) was observed in H-RHMVO and L-RHMVO fed groups, when compared the results with control and remaining group of rabbits. Statistically insignificant variation in the body and organ weight is observed in groups fed on 1.5ml of STH-OO and STH-CO. The groups fed on STH-SO had higher body and organ weights than STH-MVO, but statistically insignificant. Least weight and organ weight was increased in STH-OO group.

Plasma lipid profile

Statistically highly significant ($p < 0.005$) elevation in TGs, total cholesterol, LDL-Cholesterol and VLDL-cholesterol

,whereas lower HDL- cholesterol were observed in H-RHMVO and L- RHMVO treated groups in comparison with control group. All these parameters had significant ($p < 0.05$) variation in STH-SO fed group in comparison with control. HDL-cholesterol levels were raised in all the single time heated oils fed groups in comparison with control but statistically significant ($p < 0.05$) in STH-OO group only. Other lipid profile parameters of STH-OO, STH-CO and STH-MVO fed animals was statistically not different from control group (table 1)

Oxidative Stress and tissue damage parameters

The level of MDA, CPK, LDH, Homocysteine and CRP were elevated in H-RHMVO (3ml) and L- RHMVO (1.5ml) treated groups in comparison to STH-OO, STH-CO, STH-SO, STH-MVO and control groups, which was statistically highly ($p < 0.005$) significant. (Table 2). In all the single time heated oils fed groups these markers were statistically insignificantly changed.

DISCUSSION

The impending health benefits of several nutritive oils relative to cardiovascular disorders and potential for carcinogenicity are presently getting significant attention. In this study, organs and body weight (BW) gain was significantly high ($p < 0.005$) with 16 weeks consumption of H-RHMVO and L-RHMVO oils in comparison with control and all other groups, suggested that RHMVO use is associated with risk of obesity and CVDs even in low doses with long time consumption. These results are supported by prior animal studies reported higher BW gain with RHVO (palm and soy edible oils) than control group (Leong *et al.*, 2009; Adam *et al.*, 2008a). Results also suggest that body and organ weight gain was present in all the groups fed on oils but the BW was insignificantly gained in STH-OO and STH-CO fed animals. Supported by studies conducted in south Asian region, by Venkata and Subramanyam (2016) and Hamsi *et al* (2015). In STH-SO fed group weight gain was not only higher than STH-OO and STH-CO but also from STH-MVO group, suggesting that sunflower oil (with high PUFA content about 59%) is associated with obesity and CVDs, but safer when used in combination with other high monounsaturated FAs containing oils (olive oil with 70–80% (Tripoli *et al.*, 2005) and canola oil with 61%) (Katragadda *et al.*, 2010).

Oils containing high content of MUFA are more resistant to thermal-degradation (Lapointe *et al.*, 2006), but our study reported that in combination with oils containing high PUFA content, these oils lose this thermo protective property and exert harmful effect after repeated heating. These results are supported by studies reported that high PUFA containing oils were relatively unstable (Henna *et al.*, 2009), and associated with high risk of oxidative stress and lipid peroxidation.

Table 1: Inter group comparison of Plasma lipid profile at 16weeks (n=12)

Parameter	Control	STH-OO	STH-CO	STH-MVO	STH-SO*	L-RHMVO**	H-RHMVO**
TRIG (mg/dl)	72.65 (±6.51)	84.67 (±7.27)	104.25 (±7.64)	109.26 (±7.77)	122.55 (±4.58)	190.69 (±7.78)	270.49 (±16.21)
TC (mg/dl)	152.78 (±6.33)	191.58 (±6.14)	174.58 (±3.37)	185.99 (±3.22)	202.87 (±4.77)	358.22 (±30.77)	438.44 (±24.54)
LDL (mg/dl)	71.76 (±5.74)	76.91 (±8.70)	77.49 (±9.52)	88.40 (±5.52)	108.80 (±7.45)	280.54 (±17.26)	347.19 (±26.13)
VLDL (mg/dl)	15.19 (±1.19)	19.17 (±3.80)	21.17 (±3.80)	23.14 (±3.50)	24.17 (±3.47)	50.88 (±3.79)	74.82 (±5.94)
HDL (mg/dl)	66.54 (±7.87)	98.62 (±6.41)	76.05 (±5.43)	73.04 (±5.33)	70.35 (±6.80)	23.78 (±3.18)	17.13 (±1.67)

Table 2: Inter group comparison of Plasma tissue damage parameters at 16weeks (n=12)

Parameter	Control	STH-OO	STH-CO	STH-MVO	STH-SO	L-RHMVO**	H-RHMVO**
CPK (iu/L)	144.78 (±33.52)	168.48 (±16.01)	201.70 (±86.3)	205.50 (±81.2)	210.05 (±15.82)	704.42 (±95.33)	876.56 (±98.75)
LDH (iu/L)	239.07 (±27.62)	228.51 (±19.25)	313.82 (±9.92)	322.82 (±7.92)	352.96 (±21.04)	641.56 (±23.22)	929.67 (±14.3)
H-cys (µmol/L)	4.73 (±0.14)	4.58 (±0.05)	5.11 (±0.17)	5.71 (±0.27)	6.69 (±0.09)	8.00 (±0.33)	10.62 (±0.11)
MDA(nmol/ml)	7.96±0.25	7.96±0.31	7.97±0.33	7.97±0.34	7.99±0.45	12.02±0.66	14.09±0.79
CRP(mg/dl)	0.046 (±0.01)	0.042 (±0.02)	0.22 (±0.04)	0.32 (±0.24)	0.52 (±0.08)	2.54(±0.92)	3.99 (±1.21)

Data representation (mean ± S.D) *Values with statistically significant difference (P<0.05) and **highly Significant difference (P<0.005) in comparison with control

In accordance with results obtained by Venkata and Subramanyam (2016), the present study showed harmful effects on plasma lipoprotein profiles. The injurious effect are significantly high with RHMVO with dose-dependent hypercholesterolemia. Increasing trend in levels of serum total cholesterol in all oil treated study groups. Total-cholesterol was found significant high in H- RHMVO and L- RHMVO fed groups at comparison with control group, these results of H- RHMVO fed group is probably secondary to more than 50% cholesterol diet intake of RHMVO. Feeding with high dose RHMVO appears to quicken the rise in serum Total-Cholesterol. The consequence of chronic use of RHMVO on Total-Cholesterol levels in this study are contradictory with Hur *et al* (2005) study, reported that RHVO (corn oil) lowered the plasma Total-cholesterol levels in animals. The duration of oil feeding along with heating duration might be the major causative factors. Serum LDL-cholesterol, TG, total cholesterol and VLDL- cholesterol levels also raised in all single time heated groups but noticeably HDL-cholesterol levels also increased in all single time heated oils groups, with significant (p<0.05) elevation in HDL-levels in STH-OO group. The over expression in LDL-cholesterol levels in this present study were corresponding to study evaluated heated vegetable oil(palm oil) increase LDL-cholesterol level (Jaarin *et al.*, 2006). On the other hand, it was contradictory to

study by Adam *et al* (2008), the LDL, TG and HDL changes were not significant, used 2% cholesterol in diet that could be possible differences in the outcomes. The increased serum TG level are alike to the results of Rueda-Clausen *et al* (2007) study, reported that intake of overheated palm oil in human study increased serum Triglyceride levels. Venkata and Subramanyam (2016) also reported the similar effects of repeatedly heated oils. Few more studies reported the contradictory results, with RHVO use, as Staprans *et al* (1996), who reported no difference in serum Triglyceride levels of control and RHVO treated group. Influencing factors could be oil processing way, metabolic condition of subjects, doses along with duration of oil consumption.

Our study reported, highly significant (P<0.005) increase in MDA levels in animals fed on RHMVO, results are dose depended as MDA levels are higher with higher dose. No significant difference was found in STH-OO, STH-CO, STH-SO, STH-MVO in comparison with control. Almost Similar consequences of RHVO use were reported in another south Asian study that worked on the genotoxicity and carcinogenic impact of chronic consumption of thermally oxidized coconut oil in animal model (Srivastava *et al.*, 2010). It can hence be concluded that, consumption of RHMVO may lead to genotoxicity and neoplastic alterations (Xin-Fang *et al.*, 2012) with induced oxidative stress.

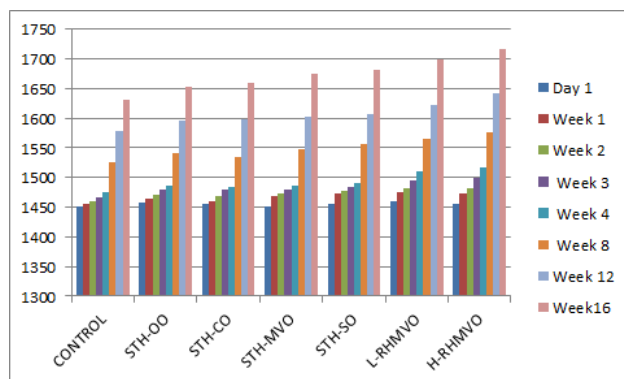


Fig. 1: Animals body weight (gm)

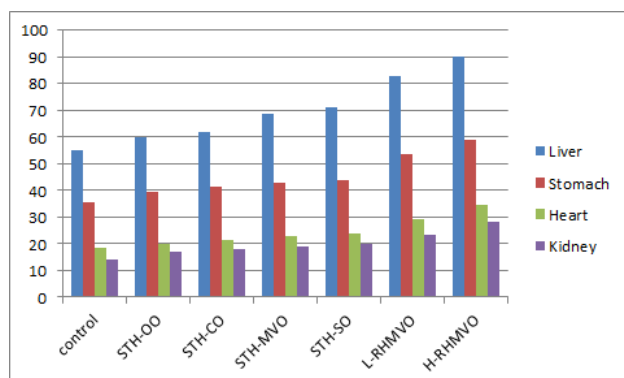


Fig. 2: Animals Organs Weight (gm)

In the present study, the LDH, CPK, homocysteine plasma levels were increased in repeated heated mixed vegetable oils groups, as compare to all other groups. The elevation in these tissue damaging markers was dose dependent, which may suggest that RHMVO may cause severe tissue damage when used in higher doses for longer duration. LDH over expression has been linked with number of disorders including tissue damages, ROS induced inflammation and endothelial cell injury (Drent *et al.*, 1996). In addition to the previous studies, it is strongly agreed that chronic intake of RHVOs is constant risk factors for CVDs development (Leong *et al.*, 2010; Ng C *et al.*, 2012).

Significantly high expression of CRP is found in both RHMVO fed groups as compare to all other oil fed groups and control. Such high value is possible indicative of developing cardiovascular disease, active inflammation, bacterial infection (Chew, 2012) and inflammatory bowel disease and other similar conditions like intestinal lymphoma and tuberculosis (Liu *et al.*, 2013). Similar results with elevated CRP levels with RHVO use are shown in study by Hamsi *et al* (2015).

These results may recommend that single time thermally processed mix vegetable oils seems to be useful with multiple benefits, but even the higher amount of MUFA cannot resistant to thermal destruction, when combined

with high PUFA containing oil, like sunflower. The heating of polyunsaturated FAs (in presence of air and moisture) has been associated with lipid peroxidation more than MUFA (Sadoudi and Ammouche, 2014), this fact has been concerned in human health protection and disease (Ramana *et al.*, 2013) and free radical induced injuries such as hypertension and metabolic changes with organ and tissue damage.

In our study potato were fried, as a common vehicle, while processing the oil to get repeatedly heated mix vegetable oil, as used in study by Jaarin *et al* (2011). Meat, fish or chicken were not used to avoid the alteration in peroxide value of oils (Kamisah *et al.*, 2012).

The oxidative stability of cooking oils while food processing at high temperatures is very important factor, which is related to their FAs constitution. canola oil and Olive oil has high oxidative stability when compared with other edible oils in different studies (Boskou, 2006; Marinova *et al.*, 2012). Sunflower oil with higher content of PUFA, is more susceptible to heat degradation in the presence of air, as reported higher degree lipid peroxidation (Sadoudia and Ammouche, 2014) which is associated with deleterious effects on human health (Ramana *et al.*, 2013). A study by Aladedunye & Przybylski (2009) evaluated that food processing (frying) at high temperature, more than 195 °C could cause PUFA isomerization from cis to trans form unhealthy isomer.

Therefore, it is recommended not to heat the individual and mixed both vegetable oils repeatedly to avoid the thermal oxidation of cooking oils and undesired health outcomes. On this basis more advance studies simulating real-life exposure to thermally oxidized oil along with food processed in it (like meat) and other environment contamination from roadside, are required.

CONCLUSION

Our study conclude that repeatedly heated oils exert health hazard when used in higher and even in daily recommended doses and may lead to inflammation, tissue necrosis, lipid per-oxidation, cardiovascular disorders and cancer. Therefore use of single time processed edible oils is recommended.

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